IDAHO BUREAU OF LABORATORIES

IDAHO DEPARTMENT OF HEALTH & WELFARE

LABORATORY CONNECTIONS

SPRING 2003

PERSPECTIVE

The events of September 11th forever changed the national perspective. This newsletter is just one of many efforts to rebuild the public health infrastructure by increasing communication among clinical laboratories. A CNN announcement will probably not herald the next incidence of biological or chemical terrorism. It may come softly, starting in isolated pockets throughout the United States and building up silently---emerging as an out-of-control public health crisis. Much information is available regarding the so called "bioterrorism agents", but history tells us to expect the unexpected. The focus of possibilities must be widened to include a variety of scenarios. A cocktail of bioterrorism agents; a genetically altered organism appearing as a newly emerging disease; or perhaps the discovery of an organism resistant to antibiotics are just some of the possibilities which need to be considered if we are to be as prepared as possible to deal with the inherent uncertainty of when, where, or how, the next bioterrorism event may occur.

This newsletter is dedicated to increasing the exchange of technical information regarding bioterrorism agents, emerging infections and the continuing increase in antibiotic resistance among microorganisms.

The Bureau of Laboratories has been able to act as a resource for some of you in the past and we would like to expand this role. Working together we can improve the public health of the people of Idaho. We ask for your input so we may be more responsive to your needs. I look forward to hearing from you.

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Bureau Chief
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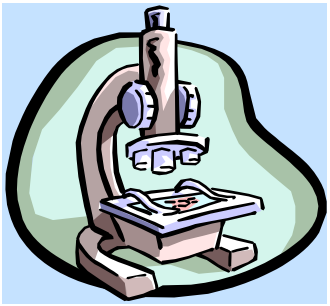

In This Issue

LABORATORY VIGILANCE PROTECTS THE PUBLIC

On the third day of a cruise catering to baby boomers, five passengers experience diarrhea, stomach pain and vomiting. The sickness spreads quickly as the ship returns to port and the passengers return home. A week later one of the passengers presents to a local ER with severe dehydration. A medical history indicates the man has a history of stomach ulcers. The doctor treats the patient and notes on his chart the patient had recently returned from a cruise which had been cut short due to illness on the ship. Approximately one week later the ER is shut down due to "winter vomiting disease."

Holiday activities find the cruise participants visiting with families including grandchildren, many of whom are in day care. New cases of an illness characterized by diarrhea, stomach pain and vomiting continue to occur in towns across the country, but since the duration of the initial disease is usually 2-3 days, many of the sick individuals do not seek medical attention.

An ER nurse notes some patients who initially presented with diarrhea, stomach pain and vomiting are presenting a second time with severe bloody diarrhea. Initial microbiological screening tests for salmonella, shigella, and *E.coli 0157:H7* are negative.

The laboratory, having limited epidemiological information, assumes this is probably Norovirus, formerly know as Norwalk virus. Is this assumption correct ? Is it possible that more than one agent is involved?.

Vigilance by laboratory personnel is critical if the health of the public is to be protected in this time of elevated concern.

CHANGES IN STAPH RESISTANCE

"Vancomycin Resistance in Staph," an article in the July 2002 issue of *Clinical Microbiology Review,* summarizes many aspects of this emerging clinical issue. The variability of vancomycin susceptibilities among subpopulations from a single isolate make detection in the laboratory difficult. At this time non-automated MIC determinations by broth or agar dilution, or by agar gradient diffusion are the "gold standard." This detection requires sufficient incubation time for expression of the resistance determinant and subsequent detectable growth. All techniques are vulnerable to problems associated with inoculum size which raises the question of heteroresistance being missed.

The NCCLS guidelines define staphylococci for which the MIC of vancomycin is ≤ 4µg/ml to be susceptible, isolates with an MIC of 8 to 16µg/ml are intermediate and those with an MIC ≥32 µg/ml are resistant.

Initial studies indicate different species of coagulase negative staph may have different vancomycin susceptibilities but the findings are not conclusive. Almost all *S. epidermidis* isolates, which represent 60% to 90% of clinical isolates, remain sensitive to vancomycin. All isolates of coagulase negative staph to date which are vancomycin *resistant are* also resistant to other antibiotics, including methicillin, quinolones, cephalosporins, and macrolides.

Srinivasan, A., J. Dick, and T. Perl. 2002 Vancomycin Resistance in Staphylococci- Clinical Microbiol. Reviews. **15**:430-438.

For a copy of this article e-mail radwins@idhw.state.id.us

STAPH RESISTANCE PROCEDURE

CDC's recommendations for detection of reduced vancomycin susceptibility *for* S. aureus are:

- MIC (broth dilution, agar dilution, or agar gradient diffusion) with a 24-hour incubation is the most accurate testing method. (Current disk diffusion procedures and rapid MIC methods do not detect intermediate strains.)
- Any *S. aureus* isolate for which the MIC ≥ 4 should be considered a candidate strain for reduced vancomycin susceptibility.
- All staph isolated from patients who fail to respond to vancomycin need to be retested in case resistance has developed during therapy.

Since vancomycin resistant *S. aureus* is very rare, the first documented case having been reported this year, it is recommended the purity of culture be confirmed and identification along with susceptibility be repeated. Notify the physician immediately of all positive Vancomycin resistant *S. aureus* (VRSA) and Vancomycin Intermediate *S. aureus* (VISA) and send the isolate to the state laboratory for con- firmation by a second method. Isolates will then be sent to CDC for addit-tional testing. See Web site:http://www.cdc.gov/ncidod/hip/Lab/FactSheet/gisa.htm

CDC. 1997. Interim guidelines for prevention and control of staphylococcal infection associated with reduced susceptibility to vancomycin. MMWR **46**:626-628, 635.

LABORATORY LIABILITY: BIOTERRORISM AGENTS

You or your administration have recently received instructions to report the possession of certain "select agents" to the Federal Government. Select agents are biological agents and toxins that pose a severe threat to animal or plant health, and animal or plant products. These instructions are the result of the Public Health Security and Bioterrorism Preparedness and Response Act of 2002. Under the Act, laboratories reporting that they do not possess such agents are exempt from further regulation. Labs that do maintain these agents, for reference purposes, will be required to register with the CDC to "certify that a facility is in compliance with specific safety and security standards" designed to prevent theft or loss of these agents. This registration must be renewed every three years. For more information and a list of select agents, see the CDC Laboratory Registration/Select Agent Transfer Web site: http://www.cdc.gov/od/sap/42cfr72.htm

The Act allows the exemption of laboratories that occasionally encounter select agents during routine diagnostic procedures, "...only if they report the identification of select agents to the Secretary (DHHW) and either promptly transfer the agent to a registered person or destroy the agent on site in accordance with regulations established by the Secretary" http://www.cdc.gov/od/sap/faq.htm

It is advised that you take stock of what is in your freezers. Categories of select agents include not only viable agents but also inactivated non-viable agents, nucleic acids from agents on the list as well as live USDA or FDA approved vaccine strains. Many labs around the United States, for example, have kept stocks of the Sterne (vaccine) strain of *Bacillus anthracis* for reference or training purposes. Under the Act, these should be destroyed or transferred to the nearest registered lab. Knowing what legal and safety precautions to take with select agents is critical in light of the anthrax events of 2001. It is important to develop policies and procedures and to train for the possibility you may see one of the agents in your laboratory someday.

Bacillus anthracis Specimen Testing by Sentinel Laboratories

SPECIMAN SELECTION		TIME and Temp		Specimen plating and processing				
		Transport	Storage	SBA	CA	Mac	Stain	Other
Cutaneous	**Vesicular Stage**: Collect fluid from intact vesicles on sterile swab	≤ 2 hr RT	≤ 24 hr RT	X	X	X	Gram Stain	Indian Ink for Capsule
	Eschar Stage: Insert swab beneath the edge and collect lesion material							
Gastrointestinal	**Stool:** Collect 5-10 g in a clean, sterile leak proof container	≤1 hr RT	≤ 24 hr 4° C	Routine stool plating media + CNA or PEA				Minimal recovery
	Blood: Collect per procedure for routine blood culture	≤2 hr RT	*	Blood Culture Bottles				Positive in late stages of disease.
Inhalation	**Sputum:** Collect expectorated specimen into a sterile, leak proof container	≤ 2 hr RT	≤ 24 hr 4°C	X	X	X	Gram Stain	Minimal recovery
	Blood: Collect per institution's procedure for routine blood culture	≤ 2 hr RT	*	Blood Culture Bottles				Positive in late stages of disease.

Abbreviations: * delayed entry depends on instrument; RT, room temperature; SBA, Sheep's Blood Agar; CA, chocolate agar; MAC, MacConkey agar; PEA, phenylethyl alcohol blood agar; CNA, colistin-nalidixic acid agar.

Bacillus anthracis Rule Out Tests by Sentinel Laboratories

- Ground glass colonies with irregular edges, some tailing, sticky consistency on sheep's blood agar.
- Rapid grower
- Non-beta hemolytic.
- Microscopically large gram positive rods.
- Non-motile either by wet prep or media.

Cultures should be held 72 hours before being reported as negative. Sporulation can begin in as little as 16 hours in the presence of oxygen. Gram stain becomes more variable as the colony ages. Motility test medium is incubated in an ambient atmosphere at 37°C for 18-24 hours. Wet Mount can be done either by transferring a loop of fresh broth culture or a loop of a suspension formed by mixing part of a 12-20 hour old colony in two drops of sterile TSB on a glass slide and cover with a cover slip. Examine under 40 X objective or under oil. **Laboratories should perform manipulations of suspicious cultures in a biological safety cabinet.**

www.bt.cdc.gov/Labissues/index.asp

Isolates which can not be ruled out should be sent immediately to the State Laboratory.

Call (208) 334-2235

If you have suspicious results or questions.

Bacillus anthracis, gram stain

The conventional preparation of iodine for Gram's stain is relatively unstable and may lose up to 60% of the available iodine in 30 days when stored at 25°C.

TRAINING CORNER

The training corner will be devoted to notifying you of training opportunities provided by the Idaho Bureau of Laboratories, announcements of professional meetings and updated information.

Two Sentinel Laboratory workshops on Bioterrorism have been held at the Idaho Bureau of Laboratories in Boise. Security restraints imposed through the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 and the Select Agent Act restricts the movement of select agents.

A dry workshop with pictures provided by CDC is scheduled in conjunction with the annual meeting of IDSCLS to be held in Idaho Falls, April 10-12, 2003.

CAP has a new BT proficiency survey designed for Sentinel A Labs. Each shipments consists of five challenges of live surrogate organisms and/or photomicrographs that approximate the characteristics of such organisms as the agents of anthrax, small pox or plague. August 4th is the date of the next shipment.

Product code :LPS
Price: $250/ 2 sets

See www.cap.org/html/lip/lps.html

Please let me know of any additional training you would like. I look forward to working with you.

Carole Morgan
BT Laboratory Training Coordinator
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IDAHO BUREAU OF LABORATORIES

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"Protecting the health and environment of the people of Idaho through testing and research"

CONNECTION NUMBERS

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Business Hours: 8:00 a.m.-5:00 p.m.
Monday – Friday (208) 334-2235

Richard Hudson, Ph.D.
Bureau Chief

Ask for microbiology section manager. If an extension does not answer, dial O for operator, and she will redirect your call.

Coeur d'Alene Branch
2195 Ironwood Court
Coeur d'Alene, ID 83814

Business Hours: 8:00 a.m.-5:00 p.m. (PST)
Monday – Friday (208) 769-1432
Ask for branch manager.

Pocatello Branch
1903 Alvin Ricken Drive
Pocatello, ID 83201

Business Hours: 8:00 a.m.-5:00 p.m.
Monday – Friday (208) 233-4341
Ask for branch manager.

Coming Next Issue

West Nile Virus

Feedback: If you have specific topics you would like covered in future issues or comments to make this newsletter more relevant, please e-mail us at radwins@idhw.state.id.us Thanks for your help.

Costs associated with this publication are available from the Idaho Department of Health and Welfare. IDHW-600-14015-7/95. Cost per unit $2.60